The Relation of Aperture to Amplification in the Selection of a Series of Microscope Objectives.

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Perhaps no subject has been more hotly discussed, or from a more purely personal standpoint, than that of the selection of a proper series of powers for the general work of the microscopist. One school insists that a large series of objectives with two or three eye-pieces is best, arguing that, as the image formed by the objective is magnified by the eye-piece, any imperfections will be correspondingly increased, and that, therefore, high power eye-pieces are undesirable and changes in amplification must be obtained by a change of objective. Hence it results that we find complete outfits advertised with three eye-pieces and a long list of objectives, four inch, two inch, one inch, two-thirds inch, one-half inch, one-eighth inch, one-twelfth inch, one-sixteenth inch, and one twentieth, or one-twenty-fifth inch. Usually the advocates of this plan are advocates of the use of objectives of narrow aperture. The objections to the plan are numerous, and among them may be mentioned the great expense of a full list of powers on this plan, and the inconvenience of changing objectives frequently. Lately the Rev. Mr. Dallinger, F. R. M. S., whose remarkable work in reference to the life histories of some of the minute flagellate monads proves him to be a most skillful microscopist, has given his views upon the subject. He can not be classed as an opponent of wide apertures, for he says, truly enough, that "much of my work could not have been done without them," but he advocates a still more elaborate series of objectives. He says: "I

have in all my special working powers three lenses of the same power, and in some cases four, and each of these in following out the life-history of an organism, say of one-three-thousandth to one-six-thousandth of an inch in length, is absolutely needed, and its place can not be supplied by any other. Thus, I have two 1-50ths, one having very low angle, and the other as great a numerical aperture as an oil immersion can provide when worked by the best makers, one 1-35ths, three 1-25ths, four 1-16ths, and so on." He also goes on in the same article to show the necessity for certain purposes of dry-working lenses of great amplifying and resolving power, because in using immersion-lenses for prolonged observation of organisms in water or other liquids there is great danger of the immersion fluid getting beyond the edge of the cover-glass, and mingling with the fluid beneath, thus destroying the minute organisms or otherwise invalidating the observations. As the cost for the series of high power objectives here mentioned, if of first quality, would be in this country more than $1,000, and the cost of the lower power objectives, stand, eye-pieces, and accessories, if selected on the same elaborate scale would probably amount to as much more, it does not require much argument to show that, if such an outfit is really necessary, the majority of workers in this country must go without, and content themselves with outfits suited only to the less delicate and elaborate investigations. In these times, when the bacteria are attracting so much attention as the putative causes of many fatal diseases, it seems a pity, if it is true, that their investigation can not be properly undertaken without an armamentarium, the first cost of which is far beyond the means of the great majority of those who are specially interested in studying them. But is it true? Can we not select a series of powers which shall cover the whole range of microscopical investigation and yet not be beyond the means of the average worker? Let us see if we can not approach the problem from the optical and mathematical side.

What is the problem? Simply to provide a microscopical outfit which shall enable us to see clearly all the details which, invisible to the unaided eye, are yet visible by the aid of the microscope. I have shown elsewhere that the normal eye can easily recognize and separate (resolve) at ten inches distance ruled lines two hundred to

the inch, and we find that, within certain limits, still finer lines become clearly visible when magnified sufficiently to bring their images seen in the microscope up to this limit of two hundred to the inch. Thus lines ten thousand to one inch when magnified fifty times, say, with a one-inch objective of 30 degrees air aperture and two-inch eye-piece, become apparently the same as lines two hundred to one inch seen with the naked eye. Judging from this alone, we might say the problem is a very simple one if our lenses are well made. All we have to do is to use those which give sufficient amplification to bring the objects up to the apparent size of lines two hundred to one inch at ten inches from the eye. But suppose we go on with our experiment and take a set of lines ruled forty thousand to one inch. To bring these to the required apparent size requires a power of two hundred diameters, which is easily obtained with the same one-inch objective by substituting a one-half inch for the two-inch. We no longer see separate lines; nor will we succeed any better with a higher objective of the same aperture as our one-inch, no matter how much we may increase the amplification. A one-tenth inch objective with the two-inch eye-piece will give us amplification of five hundred diameters—sufficient to bring the lines to an apparent scale of eighty to one inch—and yet we shall not be able to see the individual lines if our one-tenth has no more aperture than our inch. On the other hand, a one-inch objective of 50 degrees air aperture will, with the one-fourth inch eye-piece, give us an amplification of four hundred diameters and render each individual line clearly visible.

It is clear then that the rendering visible of minute details does not depend solely upon amplification, but that another element enters into the problem, and that element is the aperture of the objective. It may be well here to speak briefly of what is meant by aperture. I have elsewhere defined angular aperture as "the angular difference between the paths of the most divergent rays an objective can gather and bring to a focus." There are, however, some inconveniences connected with the use of angular aperture as the expression of the aperture of an objective. We have to express at the same time in what medium the angle is measured, as an angular aperture of 38 degrees 24 minutes in homogeneous immersion fluid is equal to

an angular aperture of 44 degrees 10 minutes in water, or of 60 degrees in air. We also find that the resolving power of an objective does not increase in direct proportion to its angle of aperture, that is, a lens of 120 degrees in air, water, or homogeneous immersion fluid has not twice the resolving power of a lens of 60 degrees in the same fluid. These and other reasons led Prof. Abbe, of Jena, to seek for some numerical expression of aperture which should be applicable to all lenses, dry or immersion, and should also bear some simple and constant relation to the most important property of aperture, i.e., resolving power. This he found by multiplying the refractive index of the medium in which the angle was measured by the sine of half the angle of aperture. The formula is:

n sine u=a, in which

n=The index of refraction of the medium;

u=Half the angle of aperture;

a=The numerical aperture of the lens.

The calculation is a simple one, readily made by anyone who has access to a table of natural sines. This gives 1.00 the numerical aperture of lens of 180 degrees angular aperture in air, or 97 degrees 31 minutes in water, or 82 degrees 17 minutes in homogeneous immersion fluid of 1.52 index of refraction. I shall hereafter use the abbreviation N. A. when speaking of numerical aperture. Among the many conveniences resulting from this method of expressing aperture, not the least is that it bears a simple numerical relation to the resolving power when light of a given wave-length is used. For ordinary purposes we use in making calculations the wave-length corresponding to Fraunhofer's line E., which is about the middle of the solar spectrum. For this wave-length the theoretical resolving power of an objective can be obtained by multiplying its N. A. by 96,400. That is, a lens of 1.00 N. A. should, at the utmost, resolve lines 96,400 to one inch, while one of .50 N. A. could not resolve lines of more than 48,200 to one inch, unless monochromatic light of shorter wave-length were used for illumination. These calculations are not at all intricate, but there is no need of making them in most cases, for a table giving N. A. from .50 to 1.52 and the corresponding angular apertures in air, water, and homogeneous immersion fluid, and the resolving power, etc., for each, is printed on the cover of

each number of *The Journal of the Royal Microscopical Society*, and has been copied in the Catalogues of the Bausch & Lomb Optical Company, of Rochester, N. Y., and so is within the reach of every one who chooses to ask for it*. Of course it is understood that the resolving power can easily be calculated for any given angular aperture in any medium quite as well as for N. A., but the discussion of the mathematical formula by which this is done would lead me beyond my purpose in this paper, and I have preferred to give the result, i. e., the numerical factor, 96,400, by which N. A. should be multiplied to obtain the theoretical resolving power.

Now, with this much by way of introduction and explanation, our work becomes simplified. Suppose we wish to know what power we need to examine a structure corresponding in fineness to lines 20,000 to one inch, and let us say that while 200 to one inch can be readily recognized by the unaided eye, yet for clear and perfectly easy recognition and examination a magnitude of 100 to one is better. It is evident, then, that we shall need an amplification of 200 diameters and an aperture of .21 N. A.,=24 degrees and 16 minutes angle of aperture in air. The question now comes up by means of what combination of objective and eye-piece shall we obtain it. And here let me say that objectives of really first-class construction only are referred to in this paper. Such objectives are so perfectly corrected that the images projected by them will bear amplification with the quarter-inch eye-piece without destroying their sharpness of outline or delicacy of detail. We can then obtain the requisite power with the one-inch objective of 30 degrees air aperture=0.26 N. A., and the half-inch eye-piece equals 200 diameters; or we can use a half-inch objective of 65 · degrees air aperture=.54 N. A. (very nearly), and the one-inch eye-piece equals 200 diameters, a quarter-inch objective of 100 degrees air aperture=.77 N. A., and the two-inch eye-piece equals 200 diameters. The amplifying power is the same in each of the three cases, and the result, so far as resolving the lines is concerned, is nearly the same. What then shall guide us in our choice? Well, first, the excellent practical rule that it is not well to use any more amplification, aperture, or illumination, than is needed for the work in hand. In relation to this particular object more than one-half of the N. A. of the half-inch objective and

* Also as an appendix to this article.

nearly two-thirds of the N. A. of the quarter-inch objective is mere surplusage, of no use whatever, but involving a great loss of working distance, diameter of field, and ease and convenience of manipulation. Suppose, however, that we wish to do the finest work possible and to know something of its probable limits. It is evident, first, that we shall need an objective of the widest possible aperture, which is, at present, about 1.42 N. A. A glance at the table in *The Journal of the Royal Microscopical Society* shows us that the utmost limit of its resolving power is 136,888 to one inch, whatever may be its amplifying power. Now to bring the image up to the apparent size of 100 to 1 inch requires an amplifying power of a little less than 1,400 diameters, which can be obtained with several combinations of eye-piece and objective. For instance:

One-quarter-in. objective and ¼-in. eye-piece=1,600 diameters.
One-sixth-in. objective and ⅓-in. eye-piece=1,800 diameters.
One-eighth-in. objective and ½-in. eye-piece=1,600 diameters.
One-sixteenth-in. objective and 1-in. eye-piece=1,600 diameters.
One-twenty-fifth-in. objective and 1-in. eye-piece=2,500 diameters.
One-fiftieth-in. objective and 2-in. eye-piece=2,500 diameters.

The 1-50-in. gives an unnecessary amount of amplification with the lowest eye-piece, as does the 1-25-in. with the next, but the 1-25 does not give enough with the lowest. According to the general principle laid down above, our choice would fall on the ¼-inch objective, but we note here that it requires our highest eye-piece to give sufficient amplification, and it is always well to have a margin, so that on the whole our choice would fall on either the ⅙ or ⅛-in. objectives, either of which gives us ample amplification with a reasonable margin of one or two higher eye-pieces in case we wish for still greater amplification of some special features. When it is recollected how enormously expensive 1-50 or 1-25-in. objectives necessarily are, how the minute size of their front lenses makes it almost impossible to construct them as perfectly as the ⅙ or ⅛-in. and that their working distance is so short as to require specially thin cover-glasses, and that after all they can show nothing that can not be as well seen with the ⅙ or ⅛-in. there seems to be no valid reason for their use. Working, then, on the general lines laid down, I have selected as a

set of powers sufficient for all the work of any microscopist the following:

One 4-inch objective of 0.10 N. A.=12° air angle, nearly.
One 1-inch objective of 0.26 N. A.=30° air angle, nearly.
One 1/6-inch objective of 0.94 N. A.=140° air angle, nearly.
One 1/8-inch objective of 1.42 N. A.

The first two to be dry-working objectives without cover correction, the third to be dry-working with cover correction, and the fourth to be a homogeneous immersion objective with cover correction, and all to be of the highest possible grade of workmanship. The stand should have a tube of such length that the standard distance of ten inches from front surface of objective to diaphragm of eye-piece can be obtained on it, and to be furnished with six eye-pieces, viz.: Two-inch, 1-inch, and ¾-inch Huyghenian, and ½, ⅓ and ¼-inch solid. The following table shows the application of these powers to all grades of work, from that which is ordinarily done with a pocket lens to the extreme limits of microscopical vision:

No. of lines to 1 inch.	N. A. required to resolve.	Equivalent angular aperture.	Amplifyi'g power needed to give apparent size of 100 to 1 inch at 10 in.	A'pli'ying power actually used.	How obtained.	
					Objective.	Eye-piece.
100.....	Less than 0.10	Less than 10 d. air.	None.	None.	Naked eye. ...	Naked eye
500. ...	Less than 0.10	Less than 10 d. air.	5	12½	4 in's of .10 N.A.	2 inches.
5,000....	Less than 0.10	Less than 10 d. air.	50	50	1 in. of .26 N.A.	2 inches.
10,000...	0.11	12 d. 38 m. air....	100	100	....	1 inch.
20,000...	0.21	24 d. 16 m. air....	200	200	....	½ inch.
30,000...	0.32	37 d. 20 m. air.. .	300	300	1-6 in. of .94 N.A.	2 inches.
40,000..	0.41	48 d. 26 m. air....	400	600	....	1 inch.
50,000...	0.52	62 d. 40 m. air...	500	600	....	1 inch.
60,000...	0.63	78 d. 08 m. air....	600	600	....	1 inch.
70,000...	0.73	93 d. 48 m. air ...	700	800	....	¾ inch.
80,000...	0.84	104 d. 17 m. air...	800	800	....	¾ inch.
90,000...	0.94	140 d. 16 m. air...	900	1,200	....	½ inch.
96,000...	1.00	180 d. air=82 d. 17 m-homogeneous im. fluid	960	1,066	1/8 in. of 1.42 N.A.	¾ inch.
100,000..	1.04	86 d. 21 m. Hom. fld.	1,000	1,066	....	¾ inch.
110,000..	1.15	About 98 d....do.	1,100	1,600	. ...	½ inch.
120,000..	1.25	About 110 d...do.	1,200	1,600	....	½ inch.
130,000..	1.35	About 125 d...do.	1,300	1,600	....	½ inch.
136,888..	1.42	About 138 d...do	1,368	1,600	....	½ inch.

It will be seen that the four-inch objective could be left out of this scheme and the work divided between the one-inch and the pocket lens or dissecting microscope, and this is probably what the majority of workers would prefer. Again it will be noted that the dry one-sixth of 0.94 N. A. might be omitted without breaking the chain of amplification or resolution, but, as Mr. Dallinger has

pointed out, a dry lens is often necessary in following out the life history of an organism growing in a fluid, and this one-sixth is amply competent for the observation of Mr. Dallinger's organism one sixth-thousandth of an inch in length, as with the one-half inch eye-piece it has amplification enough to give the organism an apparent length of one-fifth inch and resolving power enough to show parallel markings ninety thousand to the inch. Many workers, a great part of whose observations are made with powers varying from one hundred to two hundred diameters, and who yet wish at times to look up certain details requiring power up to five hundred diameters, and therefore more than .50 N. A., might put between the one-inch and one-sixth in this list a one-half inch objective of 0.64 N. A., and so the list might be varied to suit individual workers. It has not been my purpose to lay down any single set of objectives as the only proper one, but to indicate the principles on which selection should be made, and the relation of aperture to amplifying power, and to show that there is at present no good theoretical reason for the use of objectives of greater amplifying power than the one-eighth inch. It must be admitted that the strong endorsement of competent observers like the Rev. Mr. Dallinger serve to show that lenses of extremely high amplifying power, like the 1-25, 1-35 and 1-50 inch, may in special cases, be used to advantage by microscopists who have acquired the very high degree of manipulative skill necessary for their management.

NOTE 1.—The criticism of a friend who has read the manuscript of this paper reminds me that I may be charged with inconsistency in accepting as a basis the table of theoretical resolving power in reference to aperture calculated by the Helmholtz formula after what I said in reference to that formula in my presidential address before this society at Elmira, N. Y. It is to be noted, however, that there is a vast difference between accepting this formula and its results as a convenient working basis and accepting it as a final limitation and barrier to all efforts at making practical results exceed the limits of performance as laid down by theory.

NOTE 2.—The following Table of Numerical Aperture, reprinted from *The Journal of the Royal Microscopical Society*, is the one referred to in the paper.

The "APERTURE" of an optical instrument indicates its greater or less capacity for receiving rays from the object and trasmitting them to the image, and the aperture of a Microscope objective is therefore determined by the ratio between its focal length and the diameter of the emergent pencil at the plane of its emergence—that is, the utilized diameter of a single-lens objective or of the back lens of a compound objective,

This ratio is expressed for all media and in all cases by $n \sin u$, n being the refractive index of the medium and u the semi-angle of aperture. The value of $n \sin u$ for any particular case is the "*numerical aperture*" of the objective.

Numerical Aperture. ($n \sin u = a$.)	Angle of Aperture ($=2u$). *Dry* Objectives. ($n=1$.)	*Water Immersion* Objectives. ($n=1·33$.)	*Homogeneous Immersion* Objectives. ($n=1·52$.)	Illuminating Power. (a^2.)	Theoretical Resolving Power, in Lines to an inch. ($\lambda=0·5269\ \mu$ = line E.)	Penetrating Power. $\left(\frac{1}{a}\right)$
1·52	..	..	180 d. 0 m.	2·310	146,528	·658
1·50	..	..	161 d. 23 m.	2·250	144,600	·667
1·48	..	..	153 d. 39 m.	2·190	142,672	·676
1·46	..	..	147 d. 42 m.	2·132	140,744	·685
1·44	.	..	124 d. 40 m.	2·074	138,816	·694
1·42	..	..	138 d. 12 m.	2·016	136,888	·704
1·40	..	..	134 d. 10 m.	1·960	134,960	·714
1·38	..	..	130 d. 26 m.	1 904	133,032	·725
1·36	..	..	126 d. 57 m.	1·850	131,104	·735
1·34	..	..	123 d. 40 m.	1·796	129,176	·746
1·33	..	180 d. 0 m.	122 d. 06 m.	1·770	128,212	·752
1·32	..	165 d. 56 m.	120 d. 33 m.	1·742	127,248	·758
1·30	..	155 d. 38 m.	117 d 34 m	1·690	125.320	·769
1·28	..	148 d. 28 m.	114 d. 44 m.	1 638	123,392	·781
1·26	..	142 d. 39 m.	111 d. 59 m.	1·588	121,464	·794
1·24	..	137 d. 36 m.	109 d. 20 m.	1·538	119,536	·806
1·22	..	133 d. 4 m.	106 d. 45 m.	1·488	117,608	·820
1·20	..	128 d. 55 m.	104 d. 15 m.	1·440	115,680	·833
1·18	..	125 d. 3 m.	101 d. 50 m.	1·392	113,752	·847
1·16	..	121 d. 26 m.	99 d. 29 m.	1·346	111,824	·862
1·14	..	118 d. 0 m.	97 d. 11 m.	1·300	109,896	·877
1·12	..	114 d. 44 m.	94 d. 56 m.	1·254	107,968	·893
1·10	..	111 d. 36 m.	92 d. 43 m.	1·210	106,040	·909
1·08	..	108 d. 36 m.	90 d. 33 m.	1·166	104,112	·926
1·06	..	105 d. 42 m.	88 d. 26 m.	1·124	102,184	·943
1·04	..	102 d. 53 m.	86 d. 21 m.	1·082	100,256	·962
1·02	..	100 d. 10 m.	84 d. 18 m.	1·040	98,328	·980
1·00	180 d. 0 m.	97 d. 31 m.	82 d. 17 m.	1·000	96,400	1·000
0·98	157 d. 2 m.	94 d. 56 m.	80 d. 17 m.	·960	94,472	1·020
0·96	147 d. 29 m.	92 d. 24 m.	78 d. 20 m.	·922	92,544	1·042
0·94	140 d. 6 m.	89 d. 56 m.	76 d. 24 m.	·884	90,616	1·064
0·92	133 d. 51 m.	87 d. 32 m.	74 d. 30 m.	·846	88,688	1·087
0·90	128 d. 19 m.	85 d. 10 m.	72 d. 36 m.	·810	86,760	1·111
0·88	123 d. 17 m.	82 d. 51 m.	70 d. 44 m.	·774	84,832	1·136
0·86	118 d. 38 m.	80 d. 34 m.	68 d. 54 m.	·740	82,904	1·163
0·84	114 d. 17 m.	78 d. 20 m.	67 d. 6 m.	·706	80,976	1·190
0·82	110 d. 10 m.	76 d. 8 m.	65 d. 18 m.	·672	79,048	1·220
0·80	106 d. 16 m.	73 d. 58 m.	63 d. 31 m.	·640	77,120	1·250
0·78	102 d. 31 m.	71 d. 49 m.	61 d. 45 m.	·608	75,192	1·282
0·76	98 d. 56 m.	69 d. 42 m.	60 d. 0 m.	·578	73,264	1·316
0·74	95 d. 28 m.	67 d. 36 m,	58 d. 16 m.	·548	71,336	1·351
0·72	92 d. 6 m.	65 d. 32 m.	56 d. 32 m.	·518	69,408	1·389
0·70	88 d. 51 m.	63 d. 31 m.	54 d. 50 m.	·490	67,480	1·429
0·68	85 d. 41 m.	61 d. 30 m.	53 d. 9 m.	·462	65,552	1·471
0·66	82 d. 36 m.	59 d. 30 m.	51 d. 28 m.	·436	63,624	1 515
0·64	79 d. 35 m.	57 d. 31 m.	49 d. 48 m,	·410	61,696	1·562
0·62	76 d. 38 m.	55 d. 34 m.	48 d. 9 m,	·384	59,768	1·613
0·60	73 d. 44 m.	53 d. 38 m.	46 d. 30 m.	·360	57,840	1·667
0·58	70 d. 54 m.	51 d. 42 m.	44 d. 51 m.	·336	55,912	1·724
0·56	68 d. 6 m.	49 d. 48 m.	43 d. 14 m.	·314	53,984	1·786
0·54	65 d 22 m.	47 d. 54 m.	41 d. 37 m.	.292	52,056	1·852
0·52	62 d. 40 m.	46 d. 2 m.	40 d. 0 m.	·270	50,128	1·923
0·50	60 d. 0 m.	44 d. 10 m.	38 d. 24 m.	·250	48,200	2·000

Example.—The apertures of four objectives, two of which are dry, one water-immersion, and one oil-immersion, would be compared on the *angular* aperture view as follows:—106° (air), 157° (air), 142° (water), 130° (oil).

Their actual apertures are, however, as ·80 (air), ·98 (air), 1·26 (water), 1·38 (oil), or their numerical apertuers.